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HYBRID SERPINS AND DNA CODING FOR THEM

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WATERMARK PATENT & TRADEMARK ATTORNEYS, Locked Bag 5, HAWTHORN VIC 3122

(57) As a result of work done for the instant specification, the genomic clone which codes for hLS2 has been found, and its gene structure has been determined. This gene structure is utilized according to the invention for the preparation of new hybrid serpins.

CLAIM

1. A hybrid serpin as herein defined which contains amino acid part-sequences which substantially correspond to exons having the gene structure of human leuserpin-2 (hLS2).

2. A hybrid serpin as claimed in calim 1, which contains amino acid part-sequences which correspond to exons of hLS2, α_1-antitrypsin or angiotensinogen.

CONVENTION APPLICATION FOR A PATENT

611676

(1) Here insert (in full) Name or Names of Applicant or Applicants, followed by Address (es).

We (1) HOECHST AKTIENGESELLSCHAFT

of 45 Bruningstrasse, D-6230 Frankfurt/Main 80, Federal Republic of Germany

(2) Here insert Title of Invention.

hereby apply for the grant of a Patent for an invention entitled: (2)

HYBRID SERPINS AND DNA CODING FOR THEM

(3) Here insert number(s) of basic application(s)

which is described in the accompanying complete specification. This application is a Convention application and is based on the application numbered (3)

P37 09 255.3

(4) Here insert Name of basic Country or Countries, and basic date or dates

for a patent or similar protection made in (4) Federal Republic of Germany on 20th March 1987

Our address for service is Messrs. Edwd. Waters & Sons, Patent Attorneys, 50 Queen Street, Melbourne, Victoria, Australia.

DATED this 17th day of March 1988

(5) HOECHST AKTIENGESELLSCHAFT

by

D. B. Mischlewski

Registered Patent Attorney

(5) Section [illegible] Applicant [illegible] of Seal of Company and Signature of its Officers as prescribed by its Articles of Association

To:

THE COMMISSIONER OF PATENTS.

Form 3

COMMONWEALTH OF AUSTRALIA

Patents Act 1952

DECLARATION IN SUPPORT OF A CONVENTION APPLICATION UNDER PART XVI. FOR A PATENT.

In support of the Convention application made under Part XVI. of the Patents Act 1952 by HOECHST AKTIENGESELLSCHAFT of 45, Brüningstrasse, D-6230 Frankfurt/Main 80, Federal Republic of Germany for a patent for an invention entitled:
HYBRID SERPINS AND DNA CODING FOR THEM

We, Bernhard Beck, 4 Drosselweg, D-6246 Glashütten/Taunus, Franz Lapice, 2 Sandweg, D-6233 Kelkheim (Taunus), Federal Republic of Germany

do solemnly and sincerely declare as follows:

1. We are authorized by HOECHST AKTIENGESELLSCHAFT the applicant for the patent to make this declaration on its behalf.

2. The basic application as defined by Section 141 of the Act was made in the Federal Republic of Germany under No. P 37 09 255.3 **on** March 20, 1987 **by HOECHST AKTIENGESELLSCHAFT**

3. a) Hermann Ragg, 14 Am Kirchplatz, D-6233 Kelkheim (Taunus)
b) Gerald Preibisch, 18 Johann-Strauß-Straße, D-6233 Kelkheim (Taunus)
c) Friedrich Hein, 40 Erlesring, D-6234 Hattersheim am Main
d) Eugen Uhlmann, 31 Zum Talblick, D-6246 Glashütten/Taunus
e) Werner Lindenmaier, 10 Treppenkamp, D-3320 Salzgitter-Ringelheim
a) - e) Federal Republic of Germany

is/are the actual inventor(s) of the invention and the facts upon which HOECHST AKTIENGESELLSCHAFT

is entitled to make the application are as follows:

The said HOECHST AKTIENGESELLSCHAFT

is the assignee of the said
Hermann Ragg, Gerald Preibisch, Friedrich Hein, Eugen Uhlmann, Werner Lindenmaier

4. The basic application referred to in paragraph 2 of this Declaration was the first application made in a Convention country in respect of the invention the subject of the application.

DECLARED at Frankfurt/Main, Federal Republic of Germany

this 15th **day of** February **1988**

To the Commissioner of Patents

HOECHST AKTIENGESELLSCHAFT

i.V. Lapice

Prokurist / Authorized Signatory
ppa. Beck i.V. Lapice

PAT 510

611676

Form 10

COMMONWEALTH OF AUSTRALIA

PATENTS ACT 1952-69

COMPLETE SPECIFICATION

(ORIGINAL)

Class Int. Class

Application Number:
Lodged:

Complete Specification Lodged:
Accepted:
Published:

Priority :

Related Art :

Name of Applicant : HOECHST AKTIENGESELLSCHAFT

Address of Applicant : 45 Bruningstrasse, D6230 Frankfurt/Main 80, Federal Republic of Germany

Actual Inventor: HERMANN RAGG, GERALD PREIBISCH, FRIEDRICH HEIN, EUGEN UHLMANN and WERNER LINDENMAIER

Address for Service : EDWD. WATERS & SONS,
50 QUEEN STREET, MELBOURNE, AUSTRALIA, 3000.

Complete Specification for the invention entitled:

HYBRID SERPINS AND DNA CODING FOR THEM

The following statement is a full description of this invention, including the best method of performing it known to :- US

HYBRID SERPINS AND DNA CODING FOR THEM

The European Patent Application with the publication No. (EP-A) 0,190,652 discloses a human serpin which has since been given the name "leuserpin-2" (hLS2). This EP-A also reproduces the cDNA for hLS2 and points to the possibility of preparing modified genes or part-genes which produce correspondingly modified proteins in which individual amino acids have been replaced, omitted or inserted. For example, mention is made of a "building block principle" in which a part-gene I which codes for the amino acid sequence upstream of the active center is combined with a part-gene II which codes for the amino acid sequence downstream of the active center, with insertion between of a gene fragment which codes for an active center. However, the gene structure for the ~~cDNA~~ genomic DNA was not known.

As a result of work done for the instant specification, the genomic clone which codes for hLS2 has been found, and its gene structure has been determined. This gene structure is utilized according to the invention for the preparation of new hybrid serpins.

The serpins are a group of proteins which function as proteinase inhibitors in the coagulation of blood and the activation of complement and in various aspects of inflammatory reactions. The serpins belong to a protein family whose members have an amino acid homology with one another of about 20 to 35% (R.D. Doolittle, Science 222 (1983) 417-419; H. Ragg, Nucl. Acids. Res. 14 (1986) 1073-1088). The specificity of these proteinase inhibitors is determined on the one hand by an amino acid in the P1 position of the reactive center (M. Laskowski and I. Kato, Annu. Rev. Biochem. 49 91980) 593-629), as well as on the other hand by further amino acid sequences and structural elements which apparently also have an effect

on the activity of the serpins. In addition to this, in some serpins such as, for example, in angiotensinogen the N-terminal region plays an independent functional and structural role (S. Synder and R. Innis, Annu. Rev. Biochem. 48 (1979) 755-782).

The primary and tertiary structures of the serpins resemble one another (H. Loebermann et al., J. Mol. Biol. 177 (1984) 531-556; S.C. Bock et al. Biochemistry 25 (1986) 4292-4301) but, surprisingly, this is not based on a uniform gene structure, as is the case with many other protein families, for example with the globins. Of the serpin genes which have hitherto been described, only those for human α_1-antitrypsin and for rat angiotensinogen have equivalent exon/intron structures (T. Tanaka et al., J. Biol. Chem. 259 (1984) 8063-8065): in each case 5 exons are interrupted at corresponding positions by four introns. The structure of the other serpin genes hitherto known differs, however, considerably from this pattern. The human antithrombin III gene contains 6 exons (E.V. Prochownik et al., J. Biol. Chem. 260 (1985) 9608-9612), but homology can be established between only one of the 5 intron positions of α_1-antitrypsin or angiotensinogen. The gene for the human C1 inhibitor has at least 7 introns, but the location of these is as yet unknown (S.C. Bock et al., loc. cit.).

It has now been found that the hLS2 gene structure corresponds to that of α_1-antitrypsin and of (rat) angiotensinogen in respect of the number and location of the introns. This analogy is utilized according to the invention for the preparation of the hybrid serpins.

The invention is defined in its various aspects in the patent claims. Developments of the invention and preferred embodiments are explained hereinafter.

The invention is also represented in Figures 1 and 2 and in Table 1 (annex) (with, here and hereinafter, "Figure 2"

also to be understood to mean its continuation in Figure 2a):

Figure 1 shows the structure of the hLS2 gene diagrammatically. "Ex 1" to "Ex 5" represent the exons. The restriction cleavage sites are abbreviated as follows:

B	= BamHI	N	= NcoI
Bg	= BglII	Ss	= SstI
E	= EcoRI	X	= XbaI
H	= HindIII		

In the case of BglII and NcoI, only the cleavage sites necessary for construction of the hybrid gene as shown in Figure 2 are drawn.

Figure 2 shows (not true to scale) the construction of a hybrid serpin gene having the exons 1 to 4 of hLS2, represented as black bars and designated "Ex 1" to "Ex 4" as in Figure 1, and having the 3'-terminal exon of the human α_1-antitrypsin gene, depicted as shaded bars and designated "Ex α_1-AT".

Where the customary names of the restriction enzymes have been abbreviated, the explanations relating to Figure 1 apply; the following have been used additionally:

P	= PstI
S	= SalI
Sm	= SmaI
Xh	= XhoI
K	= Filling in with Klenow polymerase
S1	= Degradation with S1 nuclease
Ph	= alkaline phosphatase
L	= linker

Δ signifies cleavage sites which have been made blunt-ended by degradation or filling-in of the protruding ends.

Table 1 shows the DNA sequences of the exons and of the flanking regions of the hLS2 gene, with intron sequences being represented by small letters. The signal peptide and the signal AATAAA necessary for the formation of

correct 3'-transcript ends are underlined. The exon/intron boundaries emerge from a comparison with the known hLS2 cDNA. The arrow indicates the 5' starting point of the longest hLS2 cDNA clone hitherto found.

The term "hybrid serpin" in connection with the present invention is intended to indicate that the protein being dealt with is composed of amino acid blocks which substantially correspond to exons of hLS2 and analogous serpins having the same gene structure, and exhibits proteinase-inhibitory activity. The expression "hybrid serpins" is also intended to exclude natural products which would theoretically be obtainable by combination of identical exons from different sources. "Substantially" is intended to express the fact that the products obtained by genetic manipulation can be modified in a manner known per se, in that, for example, amino acids can be added or omitted or replaced. Modifications of these types are possible, for example, by insertion of appropriate linkers or adapters.

It is also possible to use synthetic gene fragments in the preparation according to the invention of the recombinant gene. This procedure has the advantage that it is possible to incorporate additional cleavage sites for restriction enzymes, which allow additional modifications of the encoded amino acid sequence. It is also possible in this way, for example, to modify the active centers and, in general, to produce hybrid serpins having altered substrate specificity and/or activity.

In the recombination of the exons they are expediently used with inclusion of all the necessary sequences within the introns which are necessary for splicing and other post-transcription procedures. For linking, it is possible, for example, for protruding DNA sequencesto be made blunt-ended by degradation or filling in, and thus for the exons to be connected as desired, where appropriate with insertion of synthetic oligonucleotide linkers which act as substrates for useful restriction enzymes.

Exon modules of this type can, according to the invention, be assembled in a ligase reaction in virtually any desired combination but in the correct relative orientation to one another and in the correct sequence. The hybrid genes obtained in this way can, if the promoter used is not intrinsic to serpins, be connected to a suitable eukaryotic promoter and, where appropriate, to a polyadenylation signal and, after introduction into the appropriate eukaryotic cells, their expression can be brought about there.

It is possible to use, in a manner known per se, as host/vector systems higher cells such as insect or mammalian cells. A large number of eukaryotic expression systems of these types are now known. It is also possible, where appropriate, to isolate the hybrid serpin mRNA which has formed and to employ it for the synthesis of cDNA which then - after attachment of suitable transcription signals - can be used for expression in bacteria or yeasts. If yeasts are used, it is possible to obtain glycosylated proteins typical of yeasts.

Besides the possibility of producing hybrid serpins having altered substrate specificity or activity, the invention provides an approach to the preparation of bifunctional proteins which, for example, contain the activities of angiotensin II and antitrypsin, and thus not only regulate the water balance but also have an inhibitory effect on the function of elastase.

The invention also relates to diagnostic aids which contain all or part of the genomic DNA of hLS2 to be used for the identification of genetic defects in the hLS2 gene or in diagnostic methods in which material containing human DNA or RNA is hybridized with corresponding gene probes.

The invention is explained in more detail by the examples which follow. Unless otherwise indicated, percentages are

percentages by weight.

Example 1:

Isolation of hLS2 cosmids from a human placenta gene bank

The method of W. Lindenmaier et al. (in: 35th Mosbach Colloquium 1984, "The Impact of Gene Transfer Techniques in Eukaryotic Cell Biology", Springer-Verlag Berlin, Heidelberg 1985) was used to construct a cosmid bank, with the genomic human DNA fragments which had been partially cleaved with MspI being linked to the vector pHC79-2cos/TK which had been treated with ClaI and alkaline phosphatase. The cosmid bank contains about 300,000 independent clones. 1.8×10^6 packaged cosmids were mixed with 5 ml of TM buffer (50 mM Tris-HCl, pH 7.5, 10 mM $MgSO_4$) and 5 ml of an overnight culture of E. coli DH1 which had been grown at 37°C in NZ medium (10 g/l NZ amine, 5 g/l sodium chloride, 2 g/l $MgCl_2.6\ H_2O$, 4 mg/l thiamine) containing 0.4% maltose, and were incubated at 37°C without shaking for 20 minutes. After addition of 40 ml of LB medium (10 g/l bacto tryptone (Difco), 5 g/l yeast extract (Difco), 10 g/l sodium chloride) containing 4 mg/l thiamine, the culture was shaken at 37°C for one hour.

5 ml samples of a bacterial culture of this type were distributed on autoclaved nitrocellulose membranes on the surface of an agar plate (23 x 23 cm; LB medium containing 1.4% agar and 50 µg/ml ampicillin). The plates were incubated at 37°C until the colonies had a diameter of 0.5 mm. The preparation of replica filters, the lysis and the fixation of the colonies for the hybridization were carried out by known methods (D. Hanahan and M. Meselson, Methods in Enzymology 100 (1983) 333-342, T. Maniatis et al., Molecular Cloning, Cold Spring Harbor, 1982). The filters were washed with prewashing solution (Maniatis et al., loc. cit., page 326) at 42°C for one hour. The prehybridization (4 to 6 hours) and the hybridization (16 hours at 60°C) were carried out in the following solution:

0.9 M NaCl
0.18 M Tris-HCl, pH 8.0
5 x Denhardt's solution
0.2% (w/v) SDS (sodium dodecyl sulfate)
200 µg/ml sheared and heated calf thymus DNA
200 µg/ml yeast RNA
0.5% (v/v) non-ionic detergent (Nonidet P-40; Sigma).

The probes used for the hybridization were the following restriction fragments of hLS2 cDNA (EP-A 0,190,652):

A) 1.07 kb HindIII fragment from the plasmid pH14, which embraces the 5' half of the hLS2 cDNA.

B) 0.5 kb XmnI fragment from the plasmid pL10/2. This fragment is located in the 3' half of the hLS2 cDNA (positions 1121 to 1619).

The said fragments were cut out by treatment of the relevant plasmids with the mentioned restriction enzymes, fractionated on agarose gels and then electroeluted and nick-translated ($\geq 10^8$ cpm/µg; Maniatis et al., loc. cit.).

After the hybridization, the membranes were washed, in each case for 30 minutes at room temperature, at 45°C and at 60°C, with 0.1 x SSC (1 x SSC = 0.15 M NaCl, 15 mM sodium citrate, pH 7.0) containing 0.1% SDS.

After drying, these membranes were used to expose X-ray films. Hybridizing colonies were singled out by dilution and cultured on nitrocellulose membranes, lysed and further hybridized as described. After a further singling out, there remained of the total of 750,000 analyzed colonies 3 independent hybridization-positive clones. The clone p4R hybridized only with probe A but not with probe B. Conversely, the clones p6R and p9R hybridized only with probe B but not with A.

Cosmid DNA from the clones p4R, p6R and p9R was isolated by the alkaline lysis method (Maniatis et al., loc. cit.). The DNA preparations were cut with various restriction endonucleases, fractionated on 0.7% or 1% agarose gels and transferred onto nitrocellulose membranes (E. Southern, J. Molec. Biol. 98 (1975) 503-517). Exon-containing DNA fragments of the cosmids were identified by hybridization with various radiolabelled restriction fragments of the hLS2 cDNA which are listed below:

Probe 1: HindIII/BamHI fragment which contains the region between positions 1 and 310 of the hLS2 cDNA. (The HindIII site is located in the polylinker region of the vector pUC13 in which the hLS2 cDNA is cloned).

Probe 2: BamHI fragment which embraces positions 311 to 834 of the hLS2 cDNA.

Probe 3: PvuII fragment containing the region between positions 984 and 1399.

Probe 4: XmnI fragment which embraces the region between positions 1121 and 1619.

Probe 5: EcoRI fragment which embraces the region between positions 1560 and 2081 (one of the EcoRI sites is located in the polylinker region of the vector pUC13).

All the positions specified here for restriction enzyme cleavage sites relate to the coding strand in the cDNA.

Hybridization was carried out in each case at 42°C overnight in the abovementioned hybridization buffer. The membranes were subsequently washed 2 x 15 minutes at room temperature in 2 x SSC containing 0.1% SDS and then 2 x 15 minutes at 42°C in 0.1 x SSC containing 0.1% SDS, and were dried and exposed on X-ray films. To remove the probes, the membranes were each incubated in a boiling water bath for 2 minutes and then used again for rehybridization.

For the identification of the first exon (Ex 1) the oligonucleotide having the DNA sequence

3'-CGCGGTGAAGAGTCTTTGTGTCTC-5'

(which is depicted here opposite to the customary 5'-3' direction) was synthesized by the phosphoramidite method (M.D. Matteucci et al., J. Am. Chem. Soc. 103 (1981) 3185-3191) and purified on a polyacrylamide/urea gel. The sequence of the oligonucleotide was derived from a human hLS2 cDNA sequence which was isolated from a λ gt10 cDNA bank which had been obtained by the method of T. Huynh et al., in: D.M. Glover (ed.), DNA Cloning, a Practical Approach, Vol. I, IRL Press, Oxford UK, Washington, D.C. 1985, pages 49-78. Cosmid DNA of the clone p4R was analyzed as described above using the Southern technique. The oligonucleotide having the abovementioned DNA sequence was radiolabelled using γ-^{32}P-ATP (NEN) and polynucleotide kinase (Maniatis et al., loc. cit.). The hybridization temperature was 42°C.

The membranes were washed with 6 x SSC at room temperature (2 x 15 minutes) and at 33°C (2 x 30 minutes).

On the basis of the hybridization experiments, overlapping restriction fragments of the cosmids were subcloned into the vector pUC13 by standard methods. The exons and adjacent intron regions were sequenced by the chemical degradation method of A. Maxam and W. Gilbert, Methods in Enzymology 65 (1980) 499-560. Figure 1 shows diagrammatically the structure of the hLS2 gene derived from restriction cleavage and Southern blotting as well as sequencing.

Example 2:

Subcloning of exons including flanking intron sequences of the hLS2 gene and construction of an hLS2-α_1-antitrypsin hybrid gene.

The construction of a hybrid serpin gene which can be

expressed is described by way of example and with the aid of Table 2 (and Figure 2). Of course, in place of the restriction fragments described, it is possible to use other suitable DNA fragments and other combinations of DNA fragments. In particular, it is possible to use in place of the hLS2-specific promoter region other eukaryotic promoters, for example those from another serpin gene.

Table 2

Restriction fragment	contains	Modification of the ends and subcloning
1.6 kb NcoI/EcoRI	Exon 1 of the hLS2 gene including promoter region	Filling in with Klenow fragment of DNA polymerase I from E. coli; Subcloning into the vector pUC13 which has been cleaved with SalI and treated with S1 nuclease
1.3 kb XbaI/HindIII	Exon 2 of the hLS2 gene (codes for the signal peptide inter alia)	Filling in with Klenow fragment, subcloning into the vector pUC13 which has been treated with BamHI and with S1 nuclease
0.65 kb SstI/SstI	Exon 3 of the hLS2 gene	Cleavage with S1 nuclease, subcloning into the vector pUC13 which has been cleaved with SalI and treated with S1 nuclease
1.0 kb BglII/BamHI	Exon 4 of the hLS2 gene	Filling in with Klenow fragment, subcloning into the vector pUC13 which has been treated with SmaI and alkaline phosphatase

All exon-containing restriction fragments also include any DNA sequences at the exon/intron boundaries necessary for correct splicing (B. Ruskin et al., Cell 38 (1984) 317-331; E. Keller et al., Proc. Natl. Acad. Sci. USA 81 (1984) 7417-7420; B. Wieringa et al., Cell 37 (1984) 915-925).

The isolation of the DNA fragments on agarose gels, the treatment of the fragment ends and the subcloning into the vector pUC13 are carried out by standard methods (Maniatis et al., loc. cit.). The orientation of the inserts is established by restriction cleavage, and the plasmids having the correct insertion orientation are used for the construction of the hybrid serpin gene.

Linking of exons 1 to 4 of the hLS2 gene in the correct sequence, and correct orientation of the exons with respect to one another, is likewise carried out by known methods.

Example 3:

Subcloning of the 3'-terminal exon of the human α_1-antitrypsin gene

A 1.7 kb-long XhoI/HindIII fragment having the 3'-terminal exon of the gene is isolated from a genomic clone containing the human α_1-antitrypsin gene (G. Long et al., Biochemistry 23 (1984) 4828-4837; M. Leicht et al., Nature 297 (1982) 655-659). This exon codes for, inter alia, the reactive center of the protein (G. Long et al., loc. cit.; R. Carrell et al., Nature 298 (1982) 329-334). After repair of the ends and attachment of EcoRI linkers

5'CCGAATTCGG 3'

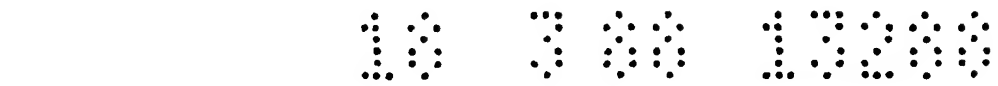
the fragment is ligated into the vector pUC13 which has been treated with EcoRI and alkaline phosphatase.

The human α_1-antitrypsin gene codes for the amino acids

methionine and serine in the reactive center of the protein. In a naturally occurring α_1-antitrypsin variant the methionyl radical has been replaced by an arginyl radical. This exchange confers antithrombotic properties on the mutated protein (M. Owen et al., New England J. Med. 309 (1983) 694-698). Mutations of this type can be introduced by known methods, for example in vitro mutagenesis (W. Kramer et al., Nucleic Acids Res. 12 (1984) 9441-9456). Exons modified in this way can, of course, also be used for the construction, according to the invention, of hybrid serpin genes.

The exons 1 to 4 of the hLS2 gene (Example 2) are linked to the 3'-terminal exon of the α_1-antitrypsin gene in a known manner (Figure 2).

Table 1



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CGTCTCTACTAAAAATACAAAAAATTAGTTGGACATGGTGGTGTGCGCCTGTAATCTCAGCCACTCAGGAGGCTGAGGCAGGAGAATCGCTTGAACTTGG

GAGACAGAGGTTGCAGTGAGCTGAGATCGCACCACTGCACTCCAGCATGGGCAGCAGCGCGAAACTCTGTCTCAAAACAAACAAACAAACAAACAAACAC

CCATAAACACAAAATGTATCACAGCCTCAGAGATCCCCACGAATGCCTAAGTGGCCCTGAATTTGGGAGGCACTGCTCAGTAATAGTCCTATCTGTCCCA

CAACAGACAGGAGTGCTGGGCTGCACCTACTGGCAACAAACACAGCAACCCTTGACTGAAGAAAGGTCCATGCCACAATCCCCTTATTCTGTAAGCCACT

AATTTTGTCCTCTCTCCTCCACCTTTCACTGAGGAACGAGCTCTTGGAAGGACAGGGACACCCGCCTAGTAGCTGAGCCAGCCACATCAGTCCTGGAGAG

CAGGTGGAGGGCAGATGCTGTGATCATCCCAGAAGAGAGGACACAGTTGGAGGCAGATGCATGGTCTCTACTTTCAGCTACCCTCAATGCAGCCTGGTCC

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TCAAAATGTCAGCTCTAAGAACTGGAGATGAGGAGCTGCAAGCCACTCTACAGTTATCAAAGGCACAGCTGAGGGGGTTTGTGGTGACCAAGCTGGTTGC

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GGG AGC AAA GGC CCG CTG GAT CAG CTA GAG AAA GGA GGG GAA ACT GCT CAG TCT GCA GAT CCC CAG TGG GAG CAG
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Leu Asn Asn Lys Asn Leu Ser Met Pro Leu Leu Pro Ala Asp Phe His Lys Glu Asn Thr Val Thr Asn Asp Trp
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                                                                     120
Lys Ser Arg Ile Gln Arg Leu Asn Ile Leu Asn Ala Lys Phe Ala Phe Asn Leu Tyr Arg Val Leu Lys Asp Gln
AAG AGC CGG ATC CAG CGT CTT AAC ATC CTC AAC GCC AAG TTC GCT TTC AAC CTC TAC CGA GTG CTG AAA GAC CAG

                                                  140
Val Asn Thr Phe Asp Asn Ile Phe Ile Ala Pro Val Gly Ile Ser Thr Ala Met Gly Met Ile Ser Leu Gly Leu
GTC AAC ACT TTC GAT AAC ATC TTC ATA GCA CCC GTT GGC ATT TCT ACT GCG ATG GGT ATG ATT TCC TTA GGT CTG
                                  160
Lys Gly Glu Thr His Glu Gln Val His Ser Ile Leu His Phe Lys Asp Phe Val Asn Ala Ser Ser Lys Tyr Glu
AAG GGA GAG ACC CAT GAA CAA GTG CAC TCG ATT TTG CAT TTT AAA GAC TTT GTT AAT GCC AGC AGC AAG TAT GAA
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Ile Thr Thr Ile His Asn Leu Phe Arg Lys Leu Thr His Arg Leu Phe Arg Arg Asn Phe Gly Tyr Thr Leu Arg
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Ser Val Asn Asp Leu Tyr Ile Gln Lys Gln Phe Pro Ile Leu Leu Asp Phe Lys Thr Lys Val Arg Glu Tyr Tyr
TCA GTC AAT GAC CTT TAT ATC CAG AAG CAG TTT CCA ATC CTG CTT GAC TTC AAA ACT AAA GTA AGA GAG TAT TAC

                                                  240
Phe Ala Glu Ala Gln Ile Ala Asp Phe Ser Asp Pro Ala Phe Ile Ser Lys Thr Asn Asn His Ile Met Lys Leu
TTT GCT GAG GCC CAG ATA GCT GAC TTC TCA GAC CCT GCC TTC ATA TCA AAA ACC AAC AAC CAC ATC ATG AAG CTC
                                  260
Thr Lys Gly Leu Ile Lys Asp Ala Leu Glu Asn Ile Asp Pro Ala Thr Gln Met Met Ile Leu Asn Cys Ile Tyr
ACC AAG GGC CTC ATA AAA GAT GCT CTG GAG AAT ATA GAC CCT GCT ACC CAG ATG ATG ATT CTC AAC TGC ATC TAC
        278
Phe Lys G
TTC AAA G gtaagaggcacctttacagttctc——(Intron B~3.7 kb)——aaccttctcataacagcctcttcctgtggcctttacag

278                                                                                  300
 ly Ser Trp Val Asn Lys Phe Pro Val Glu Met Thr His Asn His Asn Phe Arg Leu Asn Glu Arg Glu Val Val
 GA TCC TGG GTG AAT AAA TTC CCA GTG GAA ATG ACA CAC AAC CAC AAC TTC CGG CTG AAT GAG AGA GAG GTA GTT

                                                                     320
Lys Val Ser Met Met Gln Thr Lys Gly Asn Phe Leu Ala Ala Asn Asp Gln Glu Leu Asp Cys Asp Ile Leu Gln
AAG GTT TCC ATG ATG CAG ACC AAG GGG AAC TTC CTC GCA GCA AAT GAC CAG GAG CTG GAC TGC GAC ATC CTC CAG
                                                  340
Leu Glu Tyr Val Gly Gly Ile Ser Met Leu Ile Val Val Pro His Lys Met Ser Gly Met Lys Thr Leu Glu Ala
CTG GAA TAC GTG GGG GGC ATC AGC ATG CTA ATT GTG GTC CCA CAC AAG ATG TCT GGG ATG AAG ACC CTC GAA GCG
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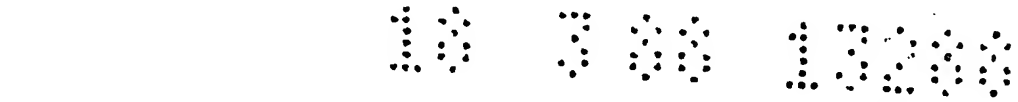

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                                360                                   369
Gln Leu Thr Pro Arg Val Val Glu Arg Trp Gln Lys Ser Met Thr Asn Ar
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tcccagatgct————————————(Intron C~1.8 kb)————————————gggccccccctttccttttctgtctag
369                                   380
  g Thr Arg Glu Val Leu Leu Pro Lys Phe Lys Leu Glu Lys Asn Tyr Asn Leu Val Glu Ser Leu Lys Leu Met
  A ACT CGA GAA GTG CTT CTG CCG AAA TTC AAG CTG GAG AAG AAC TAC AAT CTA GTG GAG TCC CTG AAG TTG ATG
                        400                                                                   417
Gly Ile Arg Met Leu Phe Asp Lys Asn Gly Asn Met Ala Gly Ile Ser Asp Gln Arg Ile Ala Ile Asp Leu
GGG ATC AGG ATG CTG TTT GAC AAA AAT GGC AAC ATG GCA GGC ATC TCA GAC CAA AGG ATC GCC ATC GAC CTG gta

accactcccttgtccacccccg————————(Intron D~0.7 kb)————————ctgacctccagaatctgacaactttcctttccaaacag

418                                                                                   440
Phe Lys His Gln Gly Thr Ile Thr Val Asn Glu Glu Gly Thr Gln Ala Thr Thr Val Thr Thr Val Gly Phe Met
TTC AAG CAC CAA GGC ACG ATC ACA GTG AAC GAG GAA GGC ACC CAA GCC ACC ACT GTG ACC ACG GTG GGG TTC ATG
                                                                  460
Pro Leu Ser Thr Gln Val Arg Phe Thr Val Asp Arg Pro Phe Leu Phe Leu Ile Tyr Glu His Arg Thr Ser Cys
CCG CTG TCC ACC CAA GTC CGC TTC ACT GTC GAC CGC CCC TTT CTT TTC CTC ATC TAC GAG CAT CGC ACC AGC TGC
                                        480
Leu Leu Phe Met Gly Arg Val Ala Asn Pro Ser Arg Ser xxx
CTG CTC TTC ATG GGA AGA GTG GCC AAC CCC AGC AGG TCC TAG AGGTGGAGGTCTAGGTGTCTGAAGTGCCTTGGGGGCACCCTCAT

TTTGTTTCCATTCCAACAACGAGAACAGAGATGTTCTGGCATCATTTACGTAGTTTACGCTACCAATCTGAATTCGAGGCCCATATGAGAGGAGCTTAGA

AACGACCAAGAAGAGAGGCTTGTTGGAATCAATTCTGCACAATAGCCCATGCTGTAAGCTCATAGAAGTCACTGTAACTGTAGTGTGTCTGCTGTTACCT

AGAGGGTCTCACCTCCCCACTCTTCACAGCAAACCTGAGCAGCGCGTCCTAAGCACCTCCCGCTCCGGTGACCCCATCCTTGCACACCTGACTCTGTCAC

TCAAGCCTTTCTCCACCAGGCCCCTCATCTGAATACCAAGCACAGAAATGAGTGGTGTGACTAATTCCTTACCTCTCCCAAGGAGGGTACACAACTAGCA

CCATTCTTGATGTCCAGGGAAGAAGCCACCTCAAGACATATGAGGGGTGCCCTGGGCTAATGTTAGGGCTTAATTTTCTCAAAGCCTGACCTTTCAAATC

CATGATGAATGCCATCAGTCCCTCCTGCTGTTGCCTCCCTGTGACCTGGAGGACAGTGTGTGCCATGTCTCCCATACTAGAGATAAATAAATGTAGCCAC

ATTTACTGTGTATCTGTTATAATTCTCTACTTTTTGAAGCTCAAATATCAAAAGCCAAATCCAAATT
```

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A hybrid serpin as herein defined which contains amino acid part-sequences which substantially correspond to exons having the gene structure of human leuserpin-2 (hLS2).

2. A hybrid serpin as claimed in calim 1, which contains amino acid part-sequences which correspond to exons of hLS2, α_1-antitrypsin or angiotensinogen.

3. A process for the paration of hybrid serpins, which comprises recombination, from exons from at least two genes which have the exon/intron structure of hLS2 and code for a serpin, of a gene which has a gene structure corresponding to hLS2, and expression of this recombinant gene in a host cell.

4. The process as claimed in claim 3, wherein the host cell is a higher eukaryotic cell.

5. A recombinant gene having a gene structure corresponding to hLS2, containing exons of serpin genes having a gene structure corresponding to hLS2.

6. A gene as claimed in claim 4, in which the exons are flanked by splice signals and branch points of the relevant introns.

7. A genomic DNA fragment containing an exon of hLS2.

8. Genomic DNA encoding hLS2.

9. A host cell transfected with DNA as claimed in any of claims 5 to 7.

10. A medicament containing a hybrid serpin as claimed in either of claims 1 or 2.

11. A diagnostic aid which contains all or part of the genomic DNA as claimed in either of claims 7 or 8.

12. A method of diagnosing human genetic defects which comprises the hybridization of human DNA with DNA as claimed in either of claims 7 or 8, or with corresponding RNA.

DATED this 22nd day of February, 1991.

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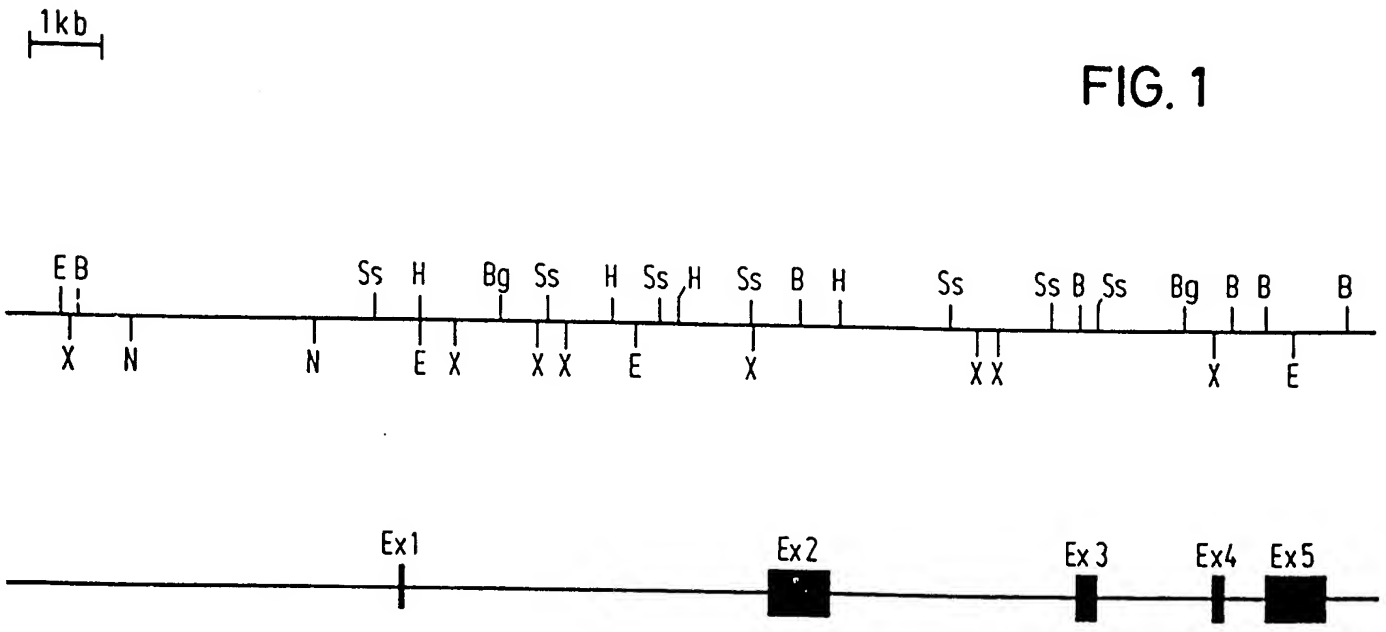
1kb
E B
Ss H Bg Ss H Ss H Ss B H Ss Ss B Ss Bg B B B
X N N E X X X E X X X X X
Ex1 Ex2 Ex3 Ex4 Ex5

FIG. 1

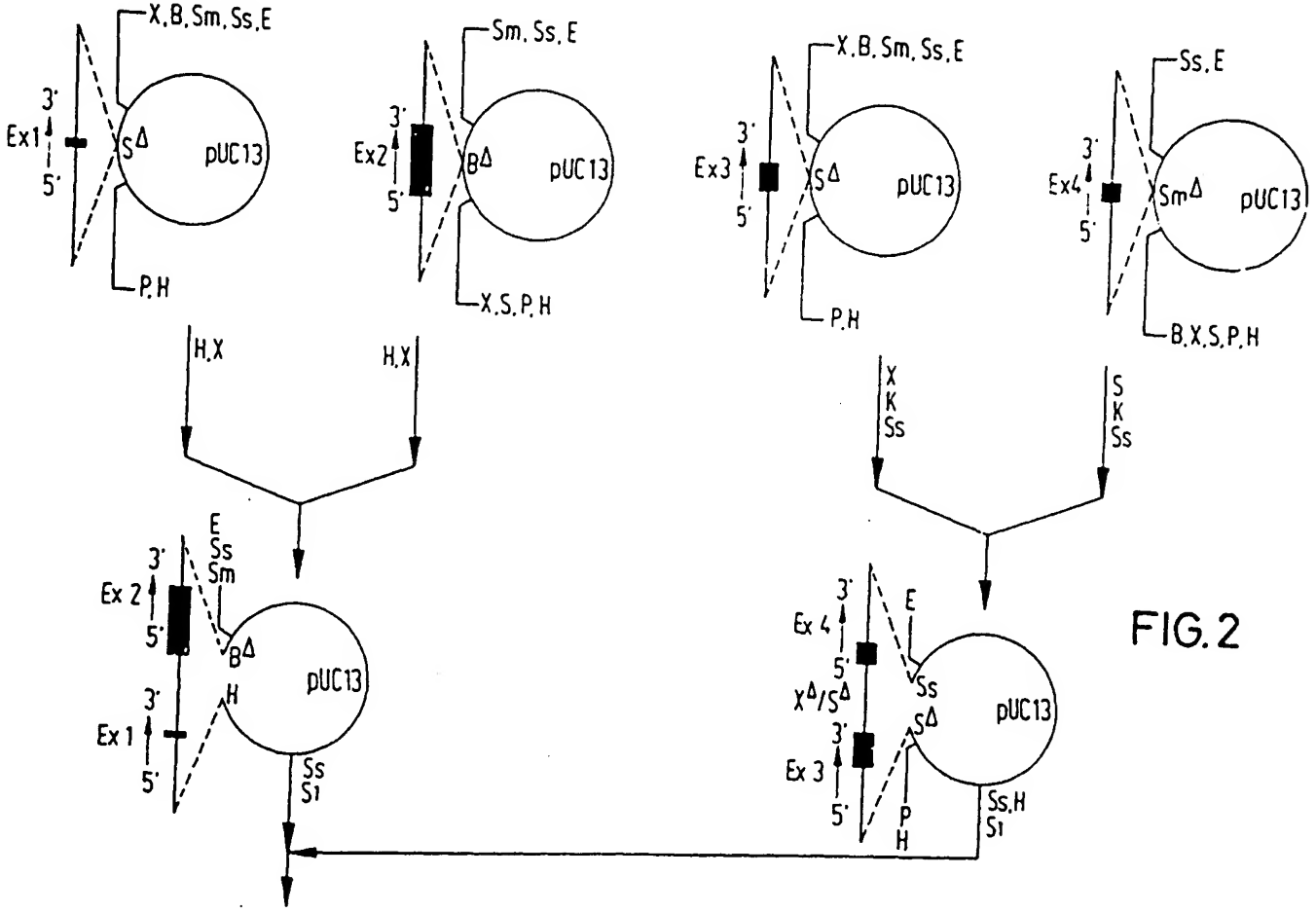
X.B.Sm.Ss.E
3'
Ex1
5'
SΔ
pUC13
P.H
Sm.Ss.E
3'
Ex2
5'
BΔ
pUC13
X.S.P.H
X.B.Sm.Ss.E
3'
Ex3
5'
SΔ
pUC13
P.H
Ss.E
3'
Ex4
5'
SmΔ
pUC13
B.X.S.P.H
H.X
H.X
X
K
Ss
S
K
Ss
E
Ss
Sm
3'
Ex 2
5'
BΔ
pUC13
H
3'
Ex 1
5'
Ss
S1
3'
Ex 4
5'
E
XΔ/SΔ
Ss
SΔ
pUC13
3'
Ex 3
5'
P
H
Ss.H
S1

FIG. 2

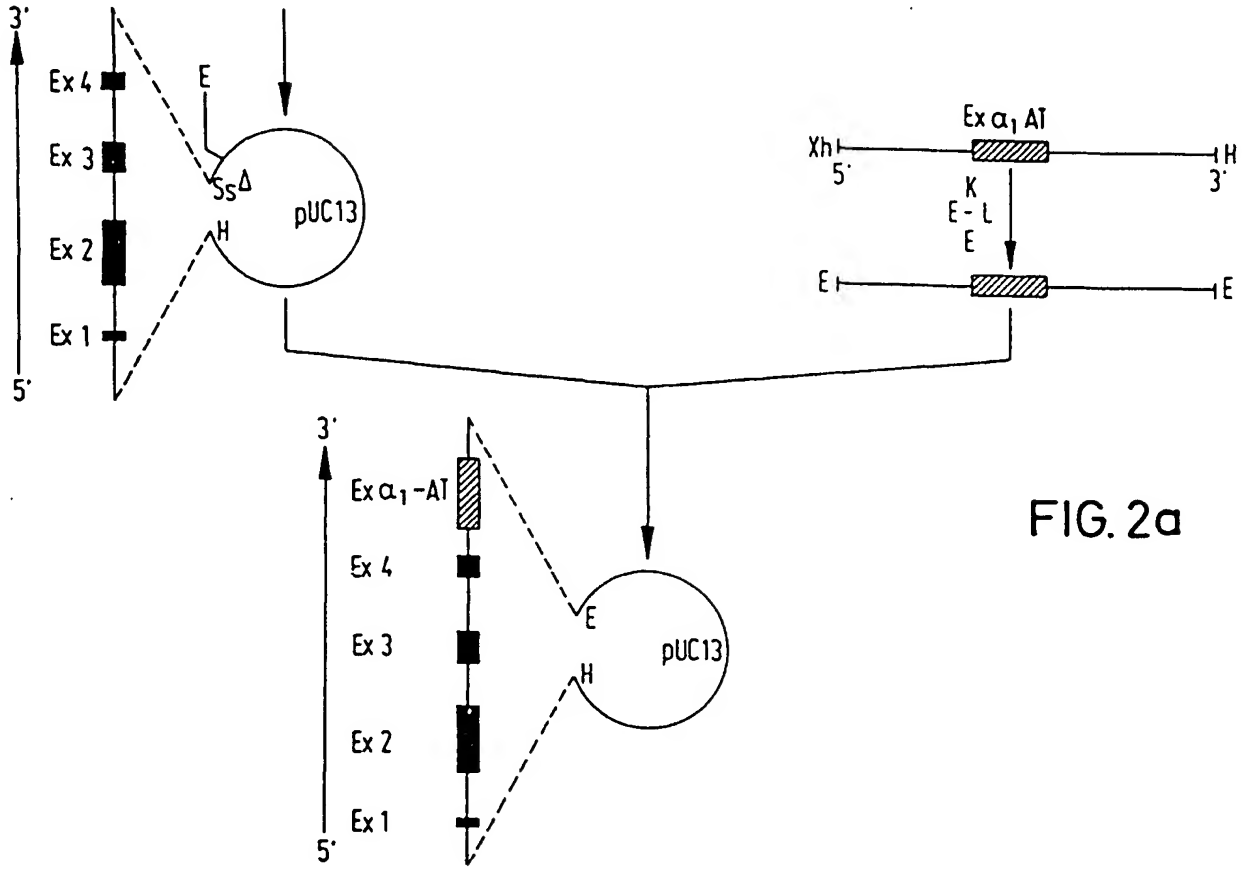
3'
Ex 4
Ex 3
Ex 2
Ex 1
5'
E
SsΔ
H
pUC13
Ex α1 AT
Xh
5'
H
3'
K
E - L
E
E
E
3'
Ex α1 -AT
Ex 4
Ex 3
Ex 2
Ex 1
5'
E
H
pUC13

FIG. 2a